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TOTAL AND ACYLATED GHRELIN LEVELS IN CHILDREN AND ADOLESCENTS WITH IDIOPATHIC SHORT STATURE AND POOR APPETITE

M. Razzaghy-Azar[1,3], M. Latifi[3], D. Ilbeygi[1,4], B. Larijani[2], M. Nourbakhsh[1,4,*]

[1]*Metabolic Disorders Research Center, Endocrinology and Metabolism Research Center,* [2]*Endocrinology and Metabolism Molecular - Cellular Sciences Institute, Tehran University of Medical Sciences,* [3]*"H. Aliasghar" Children Hospital, Iran University of Medical Sciences,* [4]*Tehran University of Medical Sciences, School of Medicine, Department of Biochemistry, Tehran, Islamic Republic of Iran*

Abstract

Context. Ghrelin is a hormone secreted primarily from stomach that can affect growth by its somatotropic and orexigenic activities.

Objective. The aim of this study was to investigate the relationship of ghrelin and growth in children and adolescents with idiopathic short stature.

Subjects and Methods. After thorough clinical examination, 56 subjects including 31 with normal weight and height and 25 with short stature were evaluated for fasting total (TG) and acylated (active) ghrelin (AG) levels. All the parameters of growth including growth hormone and IGF-1 levels, bone age and body mass index were also investigated. Appetite was also assessed and all the studied subjects were also divided into two groups, poor or good appetite.

Results. TG and AG levels were not significantly different in the two groups. There was not any significant correlation between ghrelin and parameters of growth. On the other hand, TG concentration was significantly higher in subjects with poor appetite, but AG was not significantly different.

Conclusions. The results of this study show that ghrelin is not significantly altered in idiopathic short stature. Although TG is increased in children with poor appetite its acylation is not increased concomitantly.

Key words: ghrelin, growth, short stature, appetite.

INTRODUCTION

Growth is one of the most distinctive characteristics of childhood. This process is the result of the interaction between multiple, diverse genetic, endocrine, and nutritional factors. Children normally grow at a predictable pattern; deviation from this pattern can be the first manifestation of an underlying problem which may be an endocrine or a nonendocrine disorder. Therefore, careful evaluation of growth is among the most important childcare issues (1). Many children who are referred to a paediatric endocrine clinic show deviations from the normal growth and the most frequent reason for referral to paediatric endocrinologist, after diabetes management, is for evaluation and treatment of short stature (2). Several biologic or physiologic problems are responsible for this situation and it is very important to assess and identify the underlying factors that may be associated with growth. There is often considerable overlap in the clinical presentation of patients given the diagnosis of "failure-to-thrive," or "growth failure" with some patients presenting with poor linear growth, others with poor weight gain, and some with both (3). Underweight status has been associated with higher rates of morbidity and mortality, although to a lesser extent than obesity, and higher rates of asthma, scoliosis, intestinal problems and emotional disorders were found in underweight adolescents (4).

Ghrelin is a member of motilin family peptides. It consists of 28 amino acids, is mainly secreted from stomach and has various functions (1). Ghrelin is a natural growth hormone (GH) secretagogue that increases serum GH levels (5). It also has strong orexigenic properties and increases appetite. Nevertheless, ghrelin has been shown to exert many roles including regulation of glucose homeostasis, memory & learning, food addiction and neuroprotection (6). Ghrelin is acylated by the attachment of a medium-chain fatty acid and circulates in two major forms, acylated and des-acylated ghrelin. This acylation is mandatory for its biologic effects on GH secretion and for binding at the receptor (5).

Total Ghrelin levels are decreased after consumption of a mixed meal and then are gradually increased (7). It seems that the orexigenic and metabolic properties of ghrelin are primarily mediated by its

*Correspondence to: Mitra Nourbakhsh MD, Iran University of Medical Sciences, School of Medicine, Department of Biochemistry, 1449614535, Tehran, Islamic Republic of Iran, E-mail: mitra.nourbakhsh@gmail.com

action on the hypothalamus (8). Although studies with GH secretagogues, as well as the studies with ghrelin itself, have shown a significant and reproducible GH releasing effect (9), the physiological role of circulating ghrelin in the regulation of GH release is still not fully established (5).

Mutation in ghrelin receptor (GHSR) which impairs its constitutive activity, has shown to be associated with short stature (10) and GHSR-null mice have lower IGF-I levels when compared to wild type animals (11).

Accordingly, it is possible that ghrelin can have potential impact on growth and it may play a major role in growth retardation whether short stature and/or low weight.

The aim of this study was to investigate the total and acylated ghrelin levels in children with decline in growth in the form of short stature, low weight, or both and its relationship with growth parameters, serum IGF-1 and appetite, and to compare the results with healthy controls.

MATERIALS AND METHODS

Subjects

Fifty six subjects were selected from the children and adolescents who were referred to the endocrine clinic of Hazrat-e-Aliasghar Children Hospital. All of the children had a complete history and physical exam done by a single pediatrician. The subjects were also examined by a gastroenterologist and total serum IgA and anti-tissue transglutaminase antibody (anti-tTG) were measured to rule out Celiac or any other gastroenterological disease. In order to rule out any underlying disorder, erythrocyte sedimentation rate (ESR), complete blood count (CBC) with Differential, blood electrolytes, thyroid function tests, renal function tests, urinalysis, urine culture and stool exam were performed for all the subjects.

Fifty six children and adolescents were enrolled in his study.

Exclusion criteria were as follows: 1- any specific clinical conditions including genetic disorders (e.g., Prader–Willi syndrome), endocrinopathies, dysmorphic face, and mental illness; 2- history of chronic illness, trauma, major surgery, exposure to adverse conditions, inappropriate feeding practices or failure to thrive (FTT) in the past; 3- taking mediations; 4- emotional disturbances; 5- gastrointestinal symptoms; 6- growth hormone or IGF-1 deficiency.

Inclusion criteria were as follows: 1- normal birth height and weight for gestational age; 2- suitable socioeconomic conditions. 3- age between 6 and 14 years.

All the subjects were divided into two groups: short stature group (12 boys and 13 girls) and control group (11 boys and 20 girls). Inclusion criteria for short stature group was having distance from target height (DTH) less than -1.3 SDS (12). Apart from having short stature these children and adolescents were healthy and no cause for their short stature was found. DTH calculations are explained in the next section. Inclusion criteria for normal subjects were having DTH higher than -1.2 SDS and BMI between 5th and 90th percentiles.

Pubertal staging was determined by Tanner's classification (13).

All the subjects or their parents gave informed consent, and subjects gave assent. The study conforms to the provisions of the Declaration of Helsinki in 1995 (as revised in Tokyo 2004) and was approved by the ethics committee of Endocrinology and Metabolism Research Institute and Tehran University of Medical Sciences.

Growth evaluation

Height and weight were measured with an accuracy of 0.1 cm and 0.1 kg, respectively. Body mass index (BMI) was calculated as weight (kg) divided by the square of height (m). Height, weight and BMI percentiles for age and gender of each subject were obtained from US CDC growth charts and height SDS (HSDS), weight SDS (WSDS) and BMI SDS were calculated. Height of both parents was also recorded.
Bone age was assessed by AP radiograph of left wrist according to Greulich and Pyle atlas (14). Bone age delay was calculated as bone age minus chronological age.

Target height (TH) is one of the parameters of that is directly involved in growth monitoring. TH can be calculated based on the parents' heights. Target height (TH) for boys and girls and their target height SDS (THSDS) were calculated with regard to their father's (FH) and mother's height (MH) by the following equations:

TH (boys)= [(FH+MH)/2]+6.5.

TH (girls)= [(FH+MH)/2]-6.5.

THSDS= (TH-mean adult height for age and gender) / adult height SD.

Distance from target height (DTH): HSDS - THSDS.

By comparing the HSDS of the child to its target

height, parental height and thus genetic factors are taken into account and those with familial short stature will be excluded (15). Those who had DTH less than -1.3 SDS were considered as having idiopathic short stature, according to the Dutch consensus guidelines (12).

All the subjects were also evaluated for their BMI and those with BMI lower than 5th percentile for their age and gender were compared with those with normal BMI.

Appetite evaluation

Appetite was evaluated based on Children's Eating Behavior Questionnaire (CEBQ)(16). Three items evaluating food fussiness (e.g. my child is difficult to please with meal), three items evaluating satiety responsiveness (e.g. my child gets full up easily) were derived from CEBQ. The parents were also asked to categorize their children as having good and poor appetite. According to parents' answers they were divided into good appetite and poor appetite categories.

Assays

GH stimulation test was performed after administration of Clonidine for those children who were considered short stature. IGF-1 levels were measured for all subjects. Serum GH and IGF-1 were measured by chemiluminescence sandwich assay using directly coated magnetic microplates (Diasorin Ltd, UK).

For ghrelin measurement, blood was collected after an overnight fasting in tubes containing PMSF to the final concentration of 0.1 mg/mL as a protease inhibitor. The separated serum was acidified with HCl to a final concentration of 0.05 N and was frozen at -20°C for later use. Total ghrelin was measured by an Enzyme-linked immunosorbent assay (ELISA) kit (Millipore, UK), with intra- and inter-assay CV of 5.58% and 6.24%, respectively. Acylated ghrelin was measured using a commercial ELISA kit (DRG, USA), with intra- and inter-assay CV of 6.3% and 5.4%, respectively.

Statistical analysis

SPSS 16.0 was used for statistical analyses and data presentation. Shapiro-Wilk test was used to verify the normality of all continuous variables. Statistical comparisons between the groups were performed using t tests for normally distributed variables, Mann-Whitney U-tests for non-normally distributed variables and chi-square test for categorical variables. The Pearson and Spearman correlation analyses were used to evaluate the relationship among the various continuous variables. Results are expressed as the mean ± SD or median (interquartile range); $p < 0.05$ was considered significant.

RESULTS

In this study, 56 subjects were evaluated of which 25 (44%) were male and 31 (55%) were female. Characteristics of children with idiopathic short stature and control groups are summarized in Table 1. Chronological age was not significantly different between case and control groups whereas bone age delay was more prominent in subjects with poor growth compared to controls ($p<0.01$). GH and IGF-1 levels were within normal range for all the subjects, however, IGF-1 SDS was significantly lower in cases than that in controls.

Height SDS of cases was significantly lower than that in normal subjects ($p<0.01$).

There was no significant difference in fasting total ghrelin levels between the patients and normal

Table 1. Characteristics of children with idiopathic retarded growth and normal children

	Control group	ISS	P value
Age (years)	8.70 ± 2.7	10.5 ± 3.4	0.54
Bone age (years)	7.80 ± 3.0	8.90 ± 3.9	0.46
Bone age delay (years)†	-0.8 ± 1.1	-1.6 ± 1.1	0.01
HSDS	-0.64 ± 0.94	- 1.7 ± 0.55	0.00
THSDS	-0.78 ± 0.6	-0.37 ± 0.55	0.01
DTH	0.13 ± 0.89	-1.6 ± 0.3	0.00
Weight SDS	-0.39 ±0.6	- 0.94 ± 0.58	0.00
BMI SDS	-0.22 ± 0.7	-0.37 ± 0.75	0.44
IGF-1 (ng/mL)	171.5 ± 85.5	139.5 ± 85.04	0.09
IGF-1 SDS	0.01 ± 0.57	- 0.4 ± 0.56	0.01
Total ghrelin (pg/mL)‡	466.37 (281.1-748.7)	309.1 (212.9-713.8)	0.54
Acylated ghrelin (pg/mL)‡	90.1 (51.8-166.5)	86.0 (49.3-137.9)	0.21

Values are expressed as mean ± SD; †: Bone age delay = bone age – chronological age, ‡: data for total and acylated ghrelin is expressed as median (interquartile range); *: significant difference *versus* control group; HSDS: height SDS, THSDS: target height SDS; DTH: distance from target height (HSDS – THSDS).

subjects, although ghrelin levels were slightly lower in patients than in normal subjects. Acylated ghrelin levels were not significantly different between patients and normal subjects but there was a trend towards lower acylated ghrelin levels in patients compared to normal children.

Although mean BMI SDS of case and control groups did not differ significantly, 48% of the subjects with idiopathic short stature had BMI lower than 5th percentile for their age and gender and therefore they were considered underweight. Total and acylated ghrelin levels were compared in underweight subjects and normal weight subjects and the values were not significantly different.

Total and acylated Ghrelin levels were not significantly different between male and female subjects, male and female patients or normal subjects, or prepubertal and pubertal stage.

All the subjects were divided into two groups based on their appetite. Sixty five percent of all the subjects had poor appetite, which comprised 53 % of normal subjects and 76% of patients. Poor eaters were characterized by lower food responsiveness, higher satiety responsiveness, and increased fussiness over food. The number of children with bad appetite was significantly higher in patient groups than in control group. BMI SDS was significantly lower in children with poor appetite ($p<0.05$). Total ghrelin levels were significantly higher in children with poor appetite than in children with good appetite (587.1 (285.1-906.0) *vs*. 381.4 (220.1-511.4) pg/mL, respectively; $P < 0.05$) (Fig. 1a). Comparing total ghrelin levels in normal subjects with good and poor appetite also showed significantly higher ghrelin levels in normal subjects with poor appetite than that in normal subjects with good appetite. On the other hand, acylated ghrelin levels were not significantly different in subjects with poor or appetite (92.3 (53.2-186.7) *vs*. 84.9 (44.1-135.7) pg/mL, respectively).

Neither acylated nor total ghrelin levels were significantly correlated with other variables.

DISCUSSION

The etiology of idiopathic short stature and poor growth is not well understood.

Ghrelin with its orexigenic and GH stimulating activities can be implicated in growth but whether its imbalance contributes to poor growth or not is still under debate. In the present study, no significant statistical difference was observed in total ghrelin as well as acylated or active ghrelin levels among children and adolescents with idiopathic short stature. However, the levels of this hormone were slightly lower in patients compared to healthy children although the difference was not significant. The relationship between ghrelin and growth in children has been studied previously but the results are not consistent and current data are insufficient to come to a conclusion about the involvement of ghrelin in growth retardation (3, 8, 17-

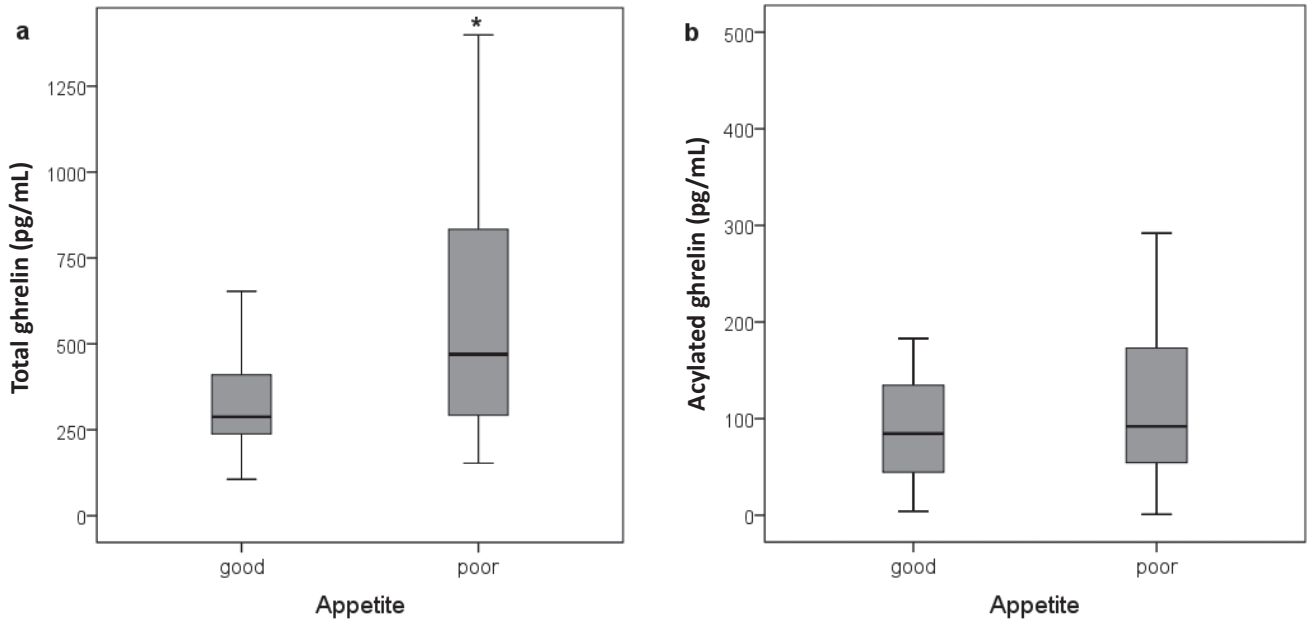

Figure 1. Median of (a) total and (b) acylated ghrelin levels in children and adolescents with good or poor appetite. Each box represents 25th and 75th percentile. Bars are minimum and maximum values. Significant difference is shown by asterisk ($p <0.05$).

19). This inconsistency may be due to the heterogeneous causes of suboptimal growth including genetic factors, GH-IGF axis abnormalities, malnutrition, and various endocrinopathies and environmental factors (8). The difference in total ghrelin levels in children with poor weight gain and suboptimal growth was significant in some studies (8, 18, 20) and not significant in others (19, 21, 22).

The relationship of acylated ghrelin and growth has been studied in two different studies. Tannenbaum *et al*. studied total and acylated ghrelin in 9 infants with FTT and 5 normal infants and showed that both acylated and total ghrelin were significantly elevated in FTT subjects compared with those observed in normally growing control infants. They proposed that increased ghrelin secretion in FTT infants is the result of an adaptive mechanism by their body attempting to increase appetite and preserve positive energy balance in response to poor nutritional status. Apart from FTT having a heterogeneous nature, their study consisted of mainly young infants and a small sample size. In comparison, in our study the age of the participants was higher and they did not have any history of FTT (23). Pinsker *et al.* also studied acylated ghrelin in 8 patients with FTT (mean age 2.2 years), 33 patients with short stature (mean age 7.9 years) and 9 patients with isolated gastrointestinal symptoms (mean age 7.3 years) which served as the control group. They found similar total ghrelin levels among different groups but higher acylated ghrelin levels in FTT patients *versus* those with isolated gastrointestinal symptoms and those with short stature. However, their study is different from the present study in that their normal subjects were not completely healthy, different groups were not age-matched, and various causes of short stature like rickets, renal tubular acidosis, growth hormone deficiency, and hypothyroidism were not ruled out (3). Thus the present study is the first one to investigate both acylated and total ghrelin levels in healthy children and adolescents with idiopathic short stature but without any present and past pathological conditions and any history of FTT.

It has been reported that weight loss as a consequence of consuming a low-calorie diet, increases ghrelin levels compared to a normal-calorie diet and this effect has been attributed to a compensatory mechanism to increase food intake (24). This is a plausible explanation with regard to decreased postprandial ghrelin levels (19). But BMI SDS was not significantly correlated with ghrelin levels in our study. Pinsker *et al*. also showed in their study that total and acylated ghrelin levels were not significantly correlated with BMI z-scores (3). Therefore it seems that ghrelin is not changed in response to BMI SDS.

Nutrition plays a fundamental role in determining the growth of individuals and suboptimal nutrition can cause growth retardation and short stature (25). Any factor that interferes with appetite and eating behavior can directly or indirectly cause growth interruption and short stature (21). Many children with a history of poor appetite and being picky eaters have been shown to have a markedly lower body height (21). In this study it was shown that children with poor appetite had significantly higher total ghrelin concentrations than that in children with good appetite. However, acylated ghrelin levels were not significantly different between these two groups. Thus it seems that although ghrelin secretion is elevated as a compensatory mechanism, its acylation is not increased concomitantly. Therefore it cannot increase appetite in low weight children and this could indirectly affect their growth. Availability of medium-chain acyl-molecules has been shown to affect ghrelin acylation (26). Poor nutrition may cause inadequacy of medium chain fatty acids for ghrelin acylation. Thus it seems possible that insufficient ghrelin acylation may be the cause of inefficient ghrelin function.

The relationship between total ghrelin and appetite has also been previously studied by Wudy *et al.* in normal children with good and poor appetite. They did not find any significant difference between the two groups, but mean BMI SDS of their whole sample was low (37th population percentile) and all of their subjects had below normal eating behavior.

In conclusion, the results of this study show that ghrelin levels are not significantly different between children and adolescents with poor growth and normal subjects. However, total ghrelin levels but not its acylated form is increased in children with poor appetite. Enhancing ghrelin acylation may be beneficial in increasing ghrelin orexigenic function in children and adolescents with poor appetite.

Conflict of interest

The authors declare that they have no conflict of interest concerning this article.

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